

## ANNEX 1

**Krieger M, Brunner T, Bischoff SC, von Tscharner V, Walz A, Moser B, Baggiolini M, Dahinden CA. Activation of human basophils through the IL-8 receptor J Immunol. 1992 Oct 15;149(8):2662-7.**

IL-8 and its structural analogs derived from blood platelets have been proposed as stimuli of IgE-independent basophil activation. In order to clarify the mechanism of action of these peptides, we examined the effects of pure IL-8, connective tissue-activating peptide III (CTAP-III), neutrophil-activating peptide 2 (NAP-2), and platelet factor 4 (PF-4) on blood basophils with and without pretreatment by IL-3, which modulates mediator release. After pretreatment with IL-3, significant histamine release was observed with 10(-8) M and 10(-7) M IL-8 and 10(-7) M NAP-2, but not with the other peptides. At higher concentrations (10(-6) M), however, all IL-8 analogs, as well as the unrelated cationic peptides poly-D-lysine, histone VS, and lysozyme, induced histamine release to variable degrees. Binding and competition studies with [<sup>125</sup>I]IL-8 revealed specific IL-8R on basophils from a patient with chronic myelogenous leukemia and normal individuals. From 3500 to 9600 receptors with a mean K<sub>d</sub> value of 0.15 nM were found on average per chronic myelogenous leukemia and normal basophil, respectively. NAP-2 weakly competed for IL-8 binding. IL-8 and, to a lesser extent, NAP-2 led to a transient rise of cytosolic free calcium concentration ([Ca<sup>2+</sup>]<sub>i</sub>), which was independent of a preexposure to IL-3. IL-8 prevented the [Ca<sup>2+</sup>]<sub>i</sub> rise induced by NAP-2, but did not influence [Ca<sup>2+</sup>]<sub>i</sub> responses to other agonists, e.g. C5a, C3a, or platelet-activating factor. IL-8 induced [Ca<sup>2+</sup>]<sub>i</sub> changes and histamine release in IL-3-primed basophils were pertussis toxin sensitive. CTAP-III or PF-4 did not compete for IL-8 binding, did not induce [Ca<sup>2+</sup>]<sub>i</sub> changes, and did not influence the [Ca<sup>2+</sup>]<sub>i</sub> response to IL-8 and NAP-2. This study shows that IL-8 and NAP-2 activate human basophils by a receptor-mediated mechanism similar to that operating in neutrophils. At high concentrations histamine release can also be induced by cationic peptides by a mechanism that does not involve the IL-8R, and probably depends on cationic interactions.

**Baggiolini M, Clark-Lewis I. Interleukin-8, a chemotactic and inflammatory cytokine. FEBS Lett. 1992 Jul 27;307(1):97-101**

Interleukin-8 (IL-8) belongs to a family of small, structurally related cytokines similar to platelet factor 4. It is produced by phagocytes and mesenchymal cells exposed to inflammatory stimuli (e.g., interleukin-1 or tumor necrosis factor) and activates neutrophils inducing chemotaxis, exocytosis and the respiratory burst. In vivo, IL-8 elicits a massive neutrophil accumulation at the site of injection. Five neutrophil-activating cytokines similar to IL-8 in structure and function have been identified recently. IL-8 and the related cytokines are produced in several tissues upon infection, inflammation, ischemia, trauma etc., and are thought to be the main cause of local neutrophil accumulation.